# Use of Plants and Rhizosphere Microorganisms for Phytoremediation

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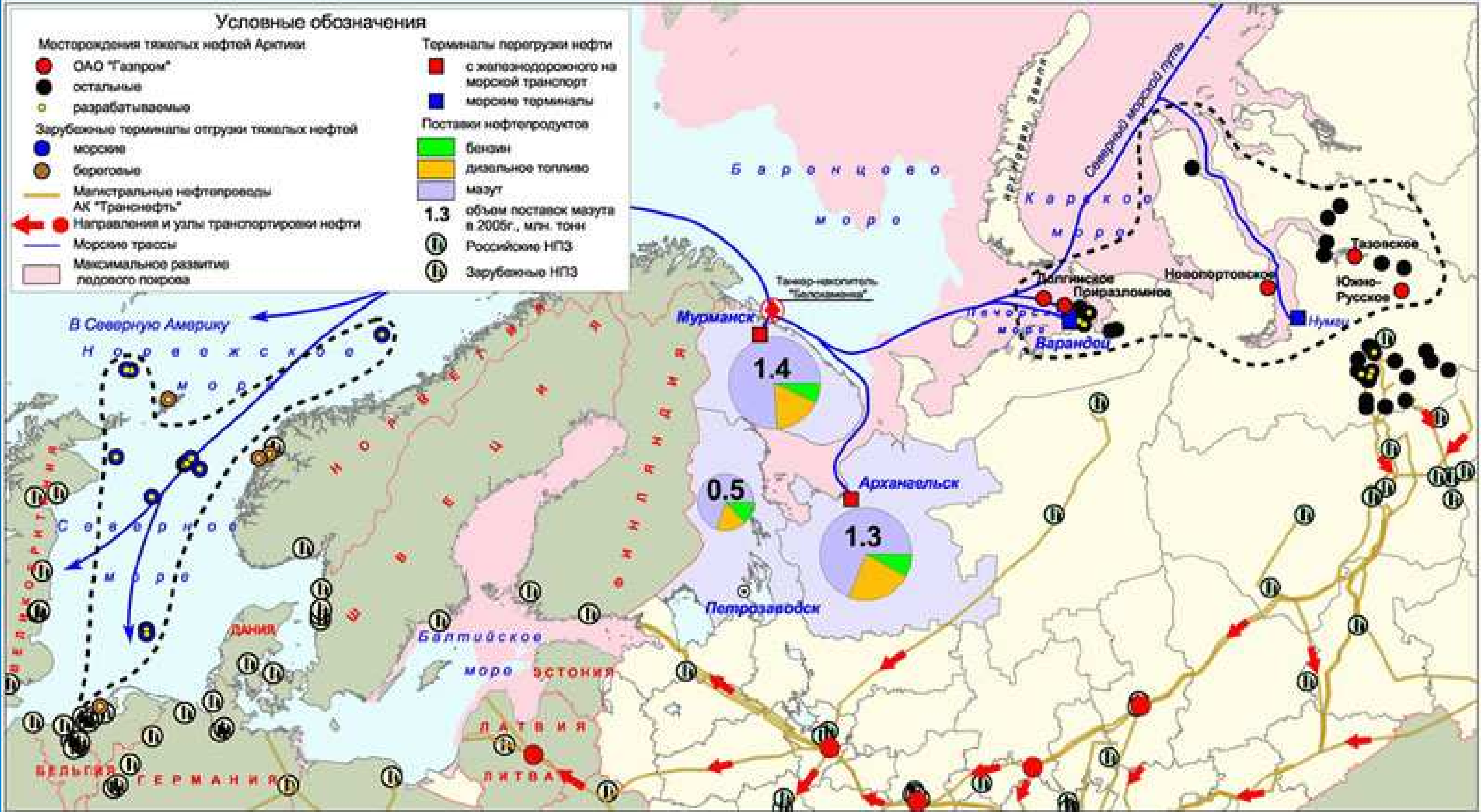
**Experts assume that average total crude-oil production in Russia is closed to 0,5 billion tons per year. Extraction, transportation and processing of big amounts of oil resulted in the contamination of soil in different regions of Russia, specifically of on vast wetlands territories in West Siberia. Total area of sites contaminated with oil and oil products is about 3500 $km^2$ . North areas biocenoses are affected with many even slight technogenic influences due to very short vegetation period.**

# Oil industrial complex in Northern Europe

http://www.dataplus.ru/Arcrev/Number_41/1_SORTOIL.html

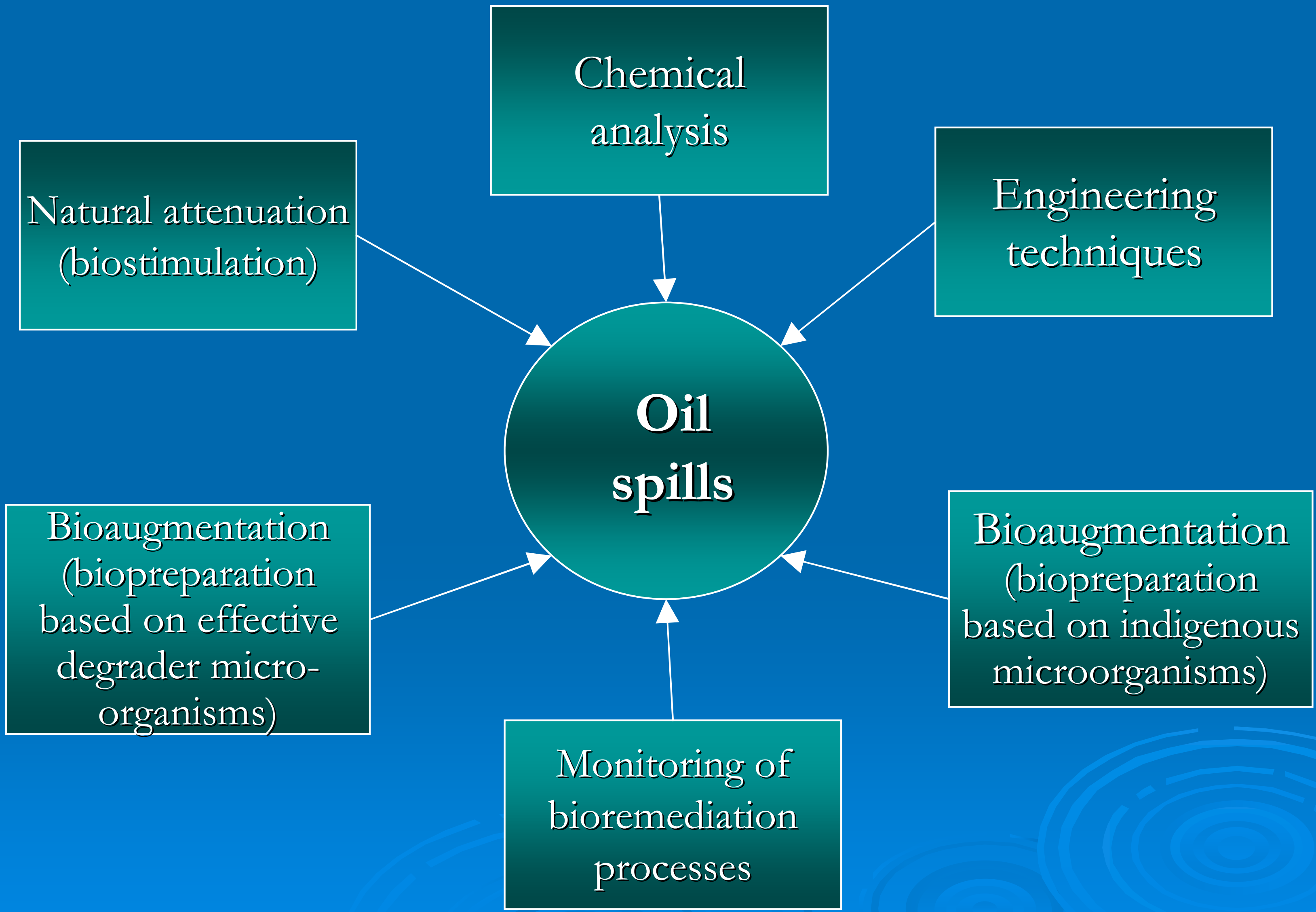


# Oil Contamination in Western Siberia

The view of oil-contaminated site.

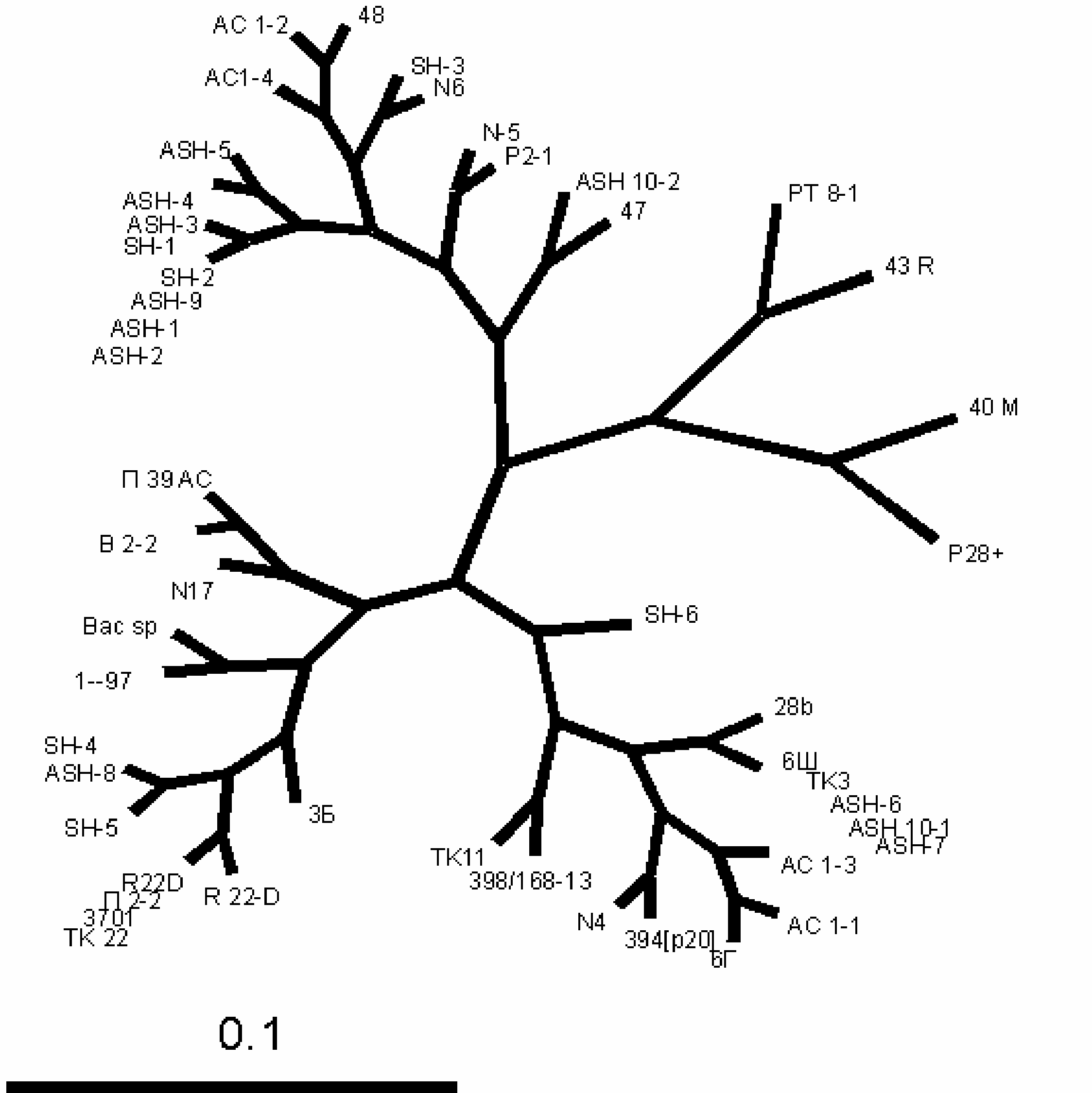
July, 1999.

# Bioremediation

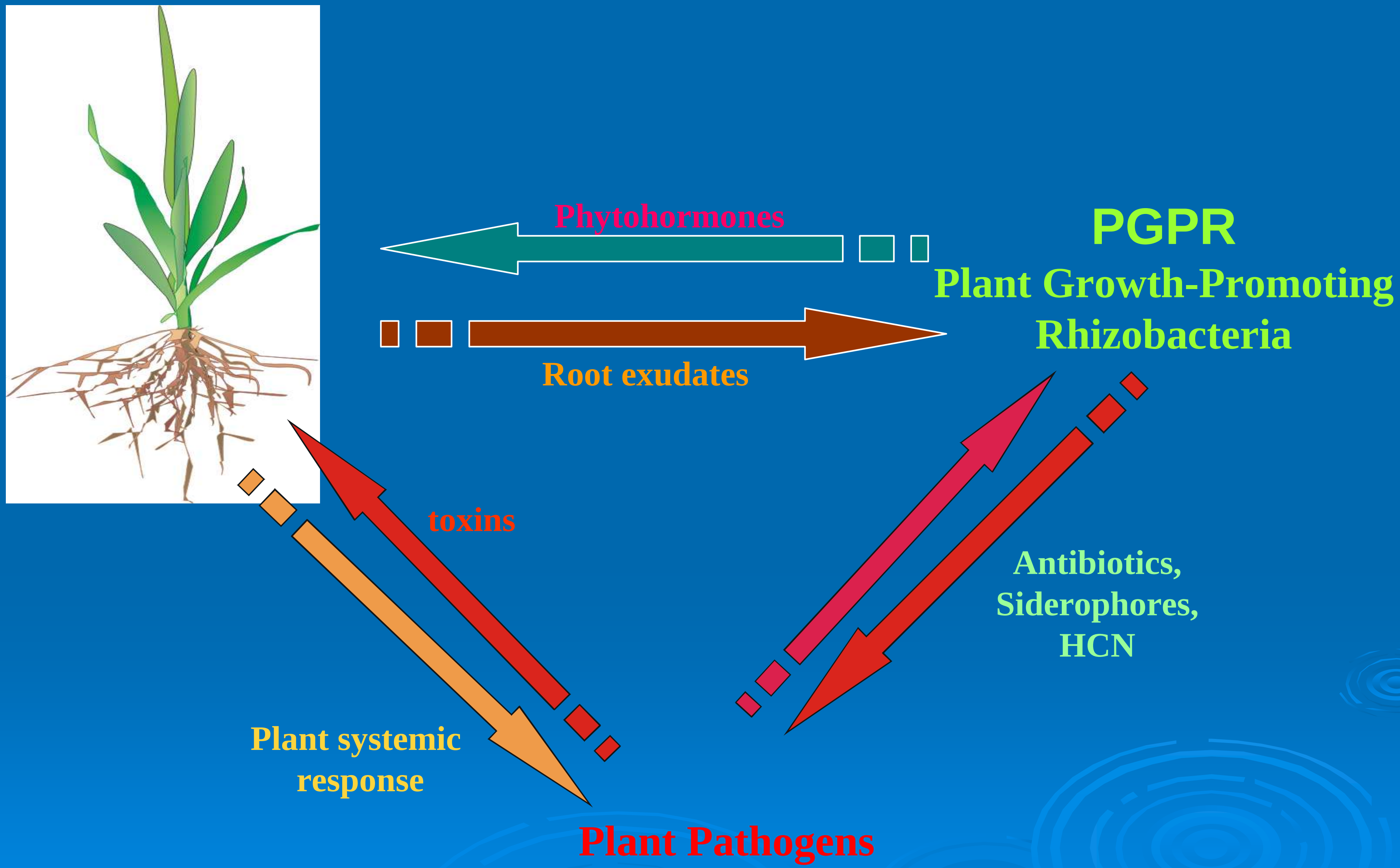


| COMPANY | LOCATION |
| --- | --- |
| Приборсервис | Tomsk, http://priborservice.tomsk.ru |
| Илма Эко | Saint-Petersburg, http://ilmaeco.ru |
| Биоойл | Novosibirsk, http://www.biooil.su/info/70 |
| СитиСтрой | Moscow |
| АгроЭкология | Ekaterinburg |
| Экойл | Tomsk |
| Экосорб | Krasnoiarsk |
| Росэкосистема | Nijnevartovsk |
| Морстрой | Saint-Petersburg, http://www.morstroy.com |
| Природа-Пермь | Perm |
| Техноспас | Moscow, http://www.tekhnospas.ru |

# Dendrogram of Oil-degrading Strains Based on Their Catabolic and Physiological Properties



# Plant-Microbial Interactions in Rhizosphere



# PSEUDOBACTERIN®-2

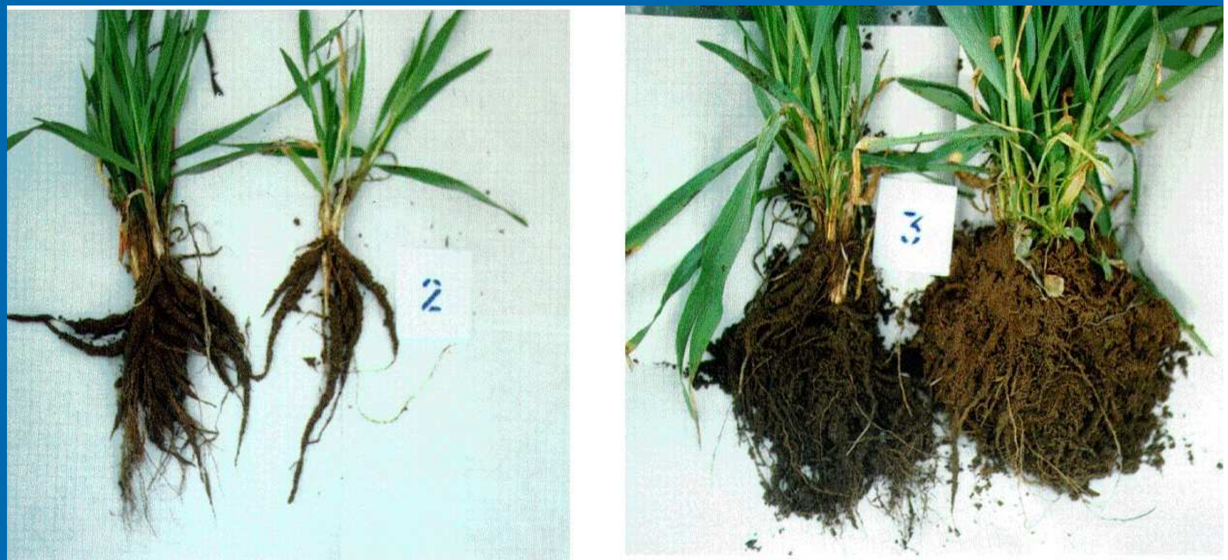
## Microbial Plant Protecting Boiopreparation is Based on Living Cells of the *Pseudomonas aureofaciens*

The author: Institute of Biochemistry and Physiology of Microorganisms Russian Academy of Sciences

### *Pseudobacterin®-2*

- \- produces phenazine compounds suppressing the growth of soil borne plant pathogenes;
- \- is highly efficient against many plant diseases;
- \- has a plant growth promoting activity;
- \- up-grades agricultural products;
- \- is combined with other pesticides;
- \- ecologically safe, harmless for people, animals, birds and insects

**The effect of preparation Pseudobacterin-2 on the growth and development of root system of winter wheat**



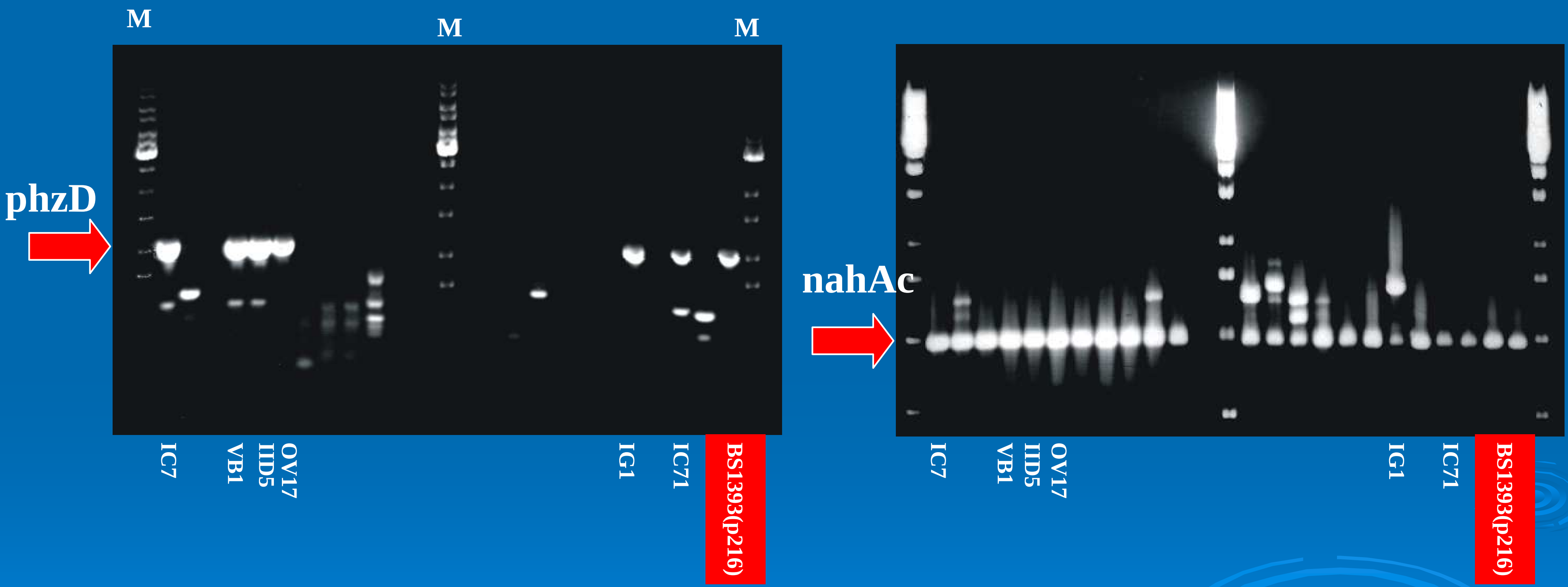

**Control** **Pseudobacterin-2**

**Examples of the increasing productivity after the treatment with Pseudobacterin-2**

**Centner (100 kg/hectare):**

| | |
|---|---|
| Cereals | 2-15 |
| Grapes | 20-37 |
| Vegetables | 30-200 |

# Wild type rhizosphere strains combining both degradative abilities and plant growth promoting properties
## (PCR analysis)




Strains harboring both phenazine antibiotic synthesis and polycyclic aromatic hydrocarbons degrading systems

# Examples of plasmids encoding the degradation of organic compounds

| *Strain* | *Plasmid* | *Substrates* | *Size (kb)* | *Inc-group* |
|---|---|---|---|---|
| **Aliphatic compounds:** | | | | |
| ***Pseudomonas oleovorans* PpG6** | **OCT** | **Octane, decane** | **500** | **P2** |
| ***Pseudomonas putida* PPU2** | **pSRQ** | **Acyclic isoprenoid (citronellol, geraniol)** | **75** | **–** |
| **Monoaromatic compounds:** | | | | |
| ***P. putida* R1** | **SAL1** | **Salicylate** | **85** | **P9** |
| ***P. putida* PpG1** | **CAM** | **Camphor** | **500** | **P2** |
| ***P. putida* PaW1** | **TOL** | **Xylene,Toluene** | **115** | **P9** |
| ***Acinetobacter calcoaceticus* RJE74** | **pWW174** | **Benzene** | **200** | **–** |
| ***Pseudomonas sp.* CIT1** | **pCIT1** | **Anililine** | **100** | **–** |
| ***P. putida* ST** | **pEG** | **Styrene** | **37** | **–** |
| ***P. putida* RE204** | **pRE4** | **Isopropyl benzene** | **105** | **–** |
| ***P. putida* NCIB9869** | **pRA500** | **3,5-xylenol** | **500** | **–** |
| ***Pseudomonas sp* CF600** | **pVI150** | **Phenol** | **–** | **P2** |
| ***P. putida* CINNP** | **pCINNP** | **Cinnamic acid** | **75** | **–** |
| ***P. putida* AC858** | **pAC25** | **3CBA** | **117** | **P1** |
| ***Ralstonia eutropha* JMP134** | **pJP4** | **2,4-D, 3CBA** | **75** | **P1** |
| ***Comamonas testosteroni* BS1310** | **pBS1010** | **p-Toluenesulfonic Acid** | **130** | **–** |
| **Polyaromatic compounds:** | | | | |
| ***P.putida* PpG7** | **NAH7** | **Naphthalene, Phenanthrene, Anthracene** | **83** | **P9** |
| ***Pseudomonas sp.* CB406** | **pWW100** | **Biphenyl** | **200** | **–** |
| ***Alcaligenes sp.* A5** | **pSS50** | **PCBs** | **53** | **P1** |
| ***Arthrobacter sp.*** | **pKF1** | **PCBs** | **80** | **–** |
| **Heterocyclic compounds:** | | | | |
| ***Pseudomonas convexa* Pcl** | **NIC** | **Nicotine, Nicotinate** | **–** | **–** |
| ***Pseudomonas alcaligenes* DBT2** | **pDBT2** | **Dibenzothiophene** | **80** | **–** |

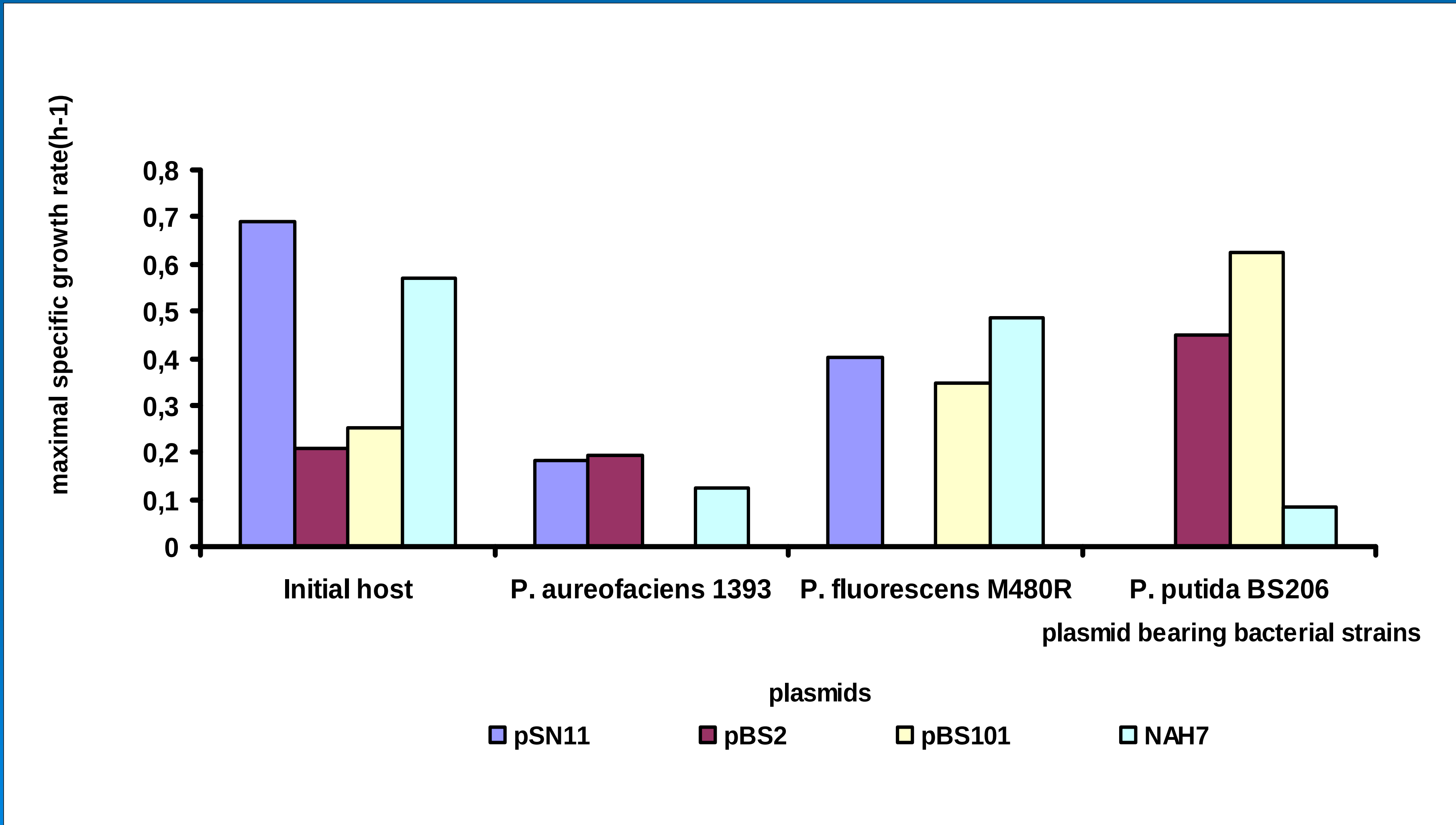
- The existing data demonstrate widespread occurrence of biodegradation plasmids throughout the bacteria. We found PAH degradation plasmids in almost 90% bacterial strains, able of PAH-destruction, including rhizosphere isolates. In contaminated soil, the rhizosphere communities undergo shifts to more abundance of microorganisms resistant to contaminants and able to degrade certain xenobiotics. We isolated strains carrying biodegradation plasmids from the rhizosphere of cereals growing in oil-contaminated regions.

# Plasmids Encoding the Degradation of Naphthalene

| Plasmid | Properties | Incompatibility group | Transfer to *P.putida* frequency | Size (kb) |
|---|---|---|---|---|
| NPL-1 | $Nah^+$ | IncP-9β | $10^{-3}$ | 100 |
| pBS2 | $Nah^+Sal^+$ | IncP-7/9β | $10^{-4}$ | 130 |
| pBS101 | $Nah^+Sal^+$ | IncP-7 | $10^{-3}$ | 50 |
| pBS240 | $Nah^+Sal^+$ | IncP-9β | $10^{-4}$ | 160 |
| pBS216 | $Nah^+Sal^+$ | IncP-9δ | $10^{-3}$ | 85 |
| pOV17 | $Nah^+Sal^+$ | IncP-9δ | $10^{-4}$ | 85 |
| pBS243 | $Nah^+Sal^+$ | IncP-7/9β | $10^{-4}$ | 160 |
| pBS213 | $Nah^+Sal^+$ | IncP-7 | $10^{-4}$ | 150 |
| pBS218 | $Nah^+Sal^+$ | IncP-7(2) | $10^{-4}$ | 160 |
| pBS219 | $Nah^+Sal^+$ | IncP-7(2) | $10^{-4}$ | 180 |
| pBS215 | $Nah^+Sal^+$ | IncP-? Not P-2, 7, 9 | $10^{-5}$ | 150 |
| pBS242 | $Nah^+Sal^+$ | IncP-? Not P-2, 7, 9 | $10^{-5}$ | 150 |
| pBS1191 | $Nah^+$ | IncP-9β | $10^{-7}$ | 100 |
| pBS1141 | $Nah^+$ | IncP-9β | $10^{-4}$ | 100 |
| pBS1181 | $Nah^+Sal^+$ | IncP-9β | $10^{-6}$ | 110 |
| pHK43 | $Nah^+Sal^+$ | IncP-7 | ND* | 100 |
| pHK72 | $Nah^+Sal^+$ | IncP-9β | ND | 85 |
| p8C | $Nah^+Sal^+$ | IncP-9β | ND | 120 |
| p15C | $Nah^+Sal^+$ | IncP-9β | ND | 120 |
| p24C | $Nah^+Sal^+$ | IncP-9β | ND | 120 |
| p25C | $Nah^+Sal^+$ | IncP-9β | ND | 120 |

ND – not determined.

# Specific Growth Rates of Plasmid Bearing Bacterial Strains in Batch Culture on Naphthalene



# Effect of degradative plasmids on interaction of *PGPR* strains and mustard plants (*Brassica juncea L.*) under gnotobiotic conditions in sand supplemented with naphthalene (1g/kg).

| *P. putida* 53a(pOV17) | *P. aureofaciens* OV17(pOV17) | *P. putida* 53a(pBS216) | no bacteria with naphthalene | no bacteria no naphthalene |
|---|---|---|---|---|

**Treatment of seedlings with plasmid-bearing rhizobacteria led to a protective effect from naphthalene.**

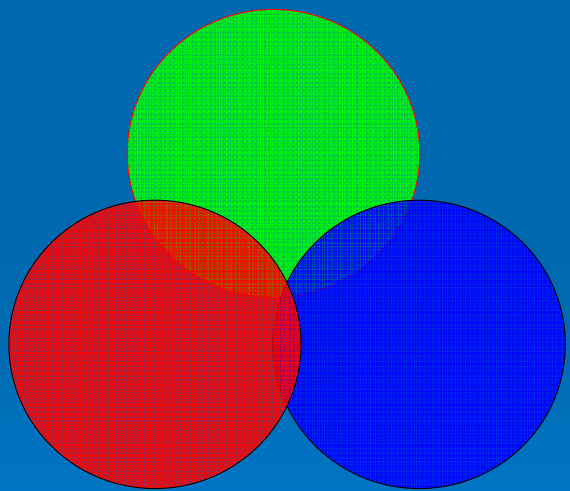
**The exception was the seedlings treated with *P. putida* 53a(pBS216).**

**In this case no seed germination was observed**

# Use of Plants and PGPR for Phytoremediation of co-contaminated soils

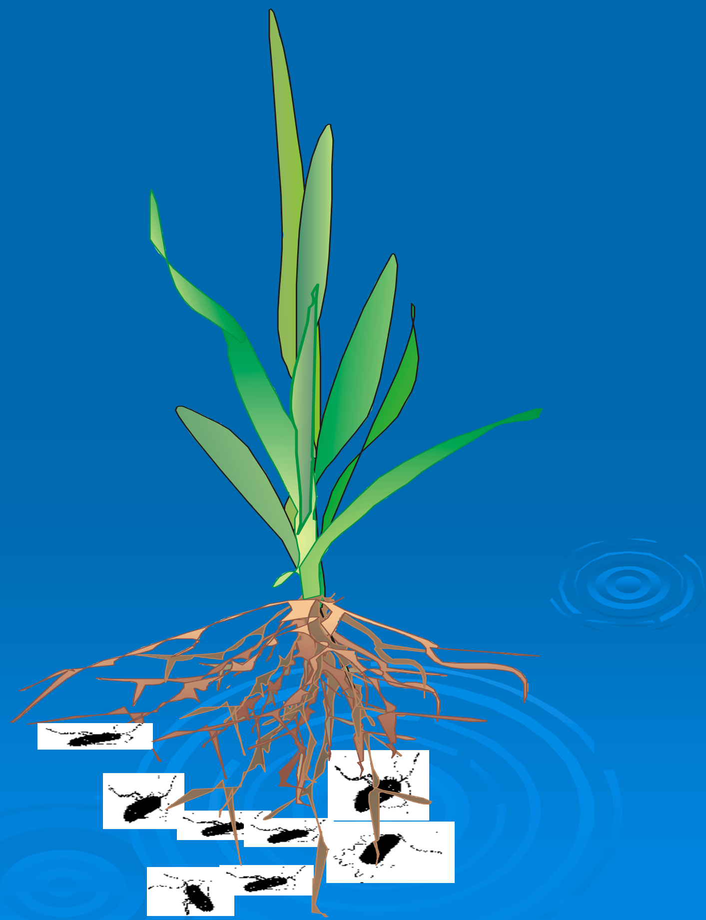
Construction of plasmid-bearing PGPR *Pseudomonas* → Inoculation of seeds

**Stimulation of plant growth and protection against phytopathogenic fungi**

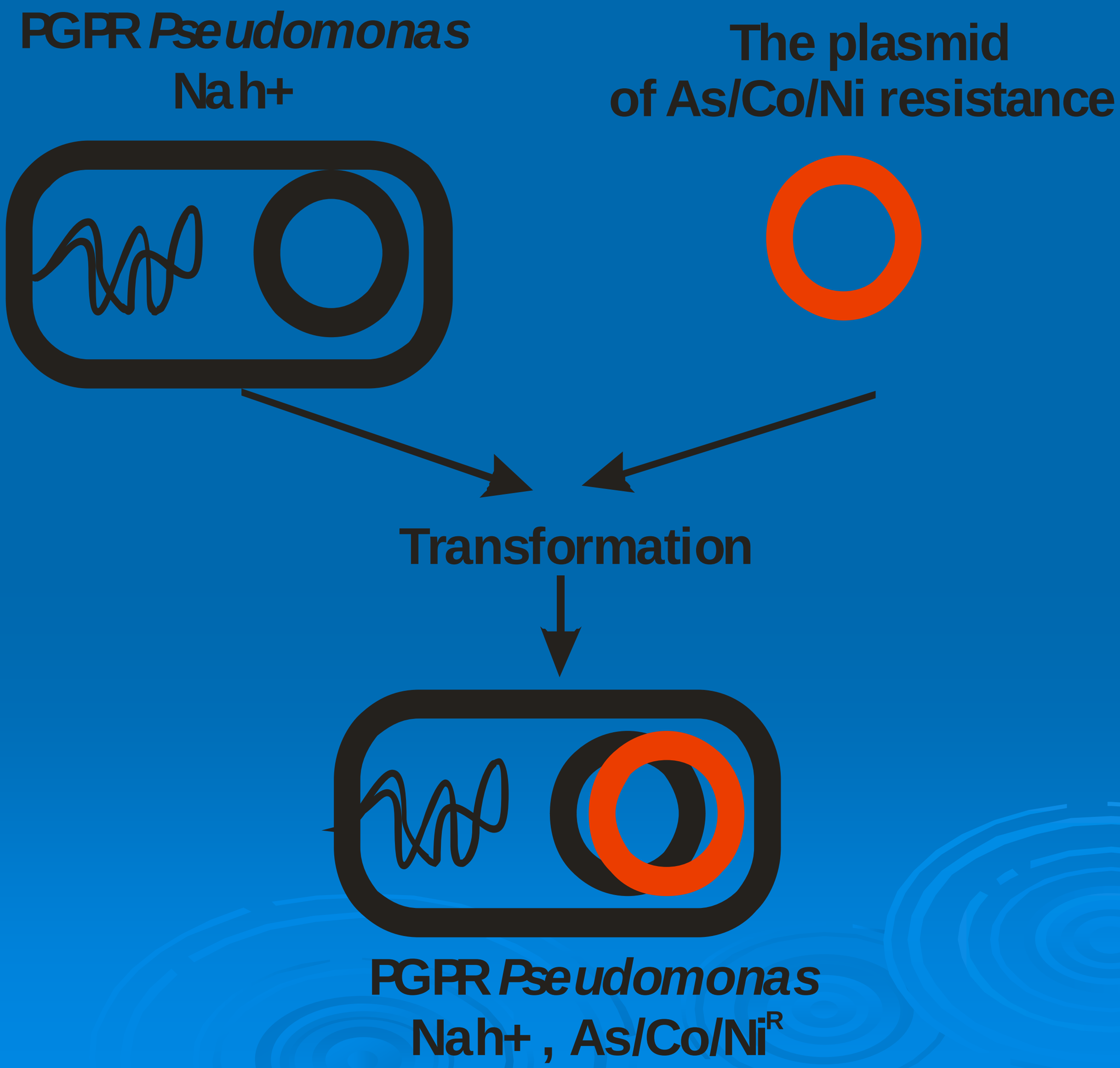


**Heavy metal/arsenic resistance**

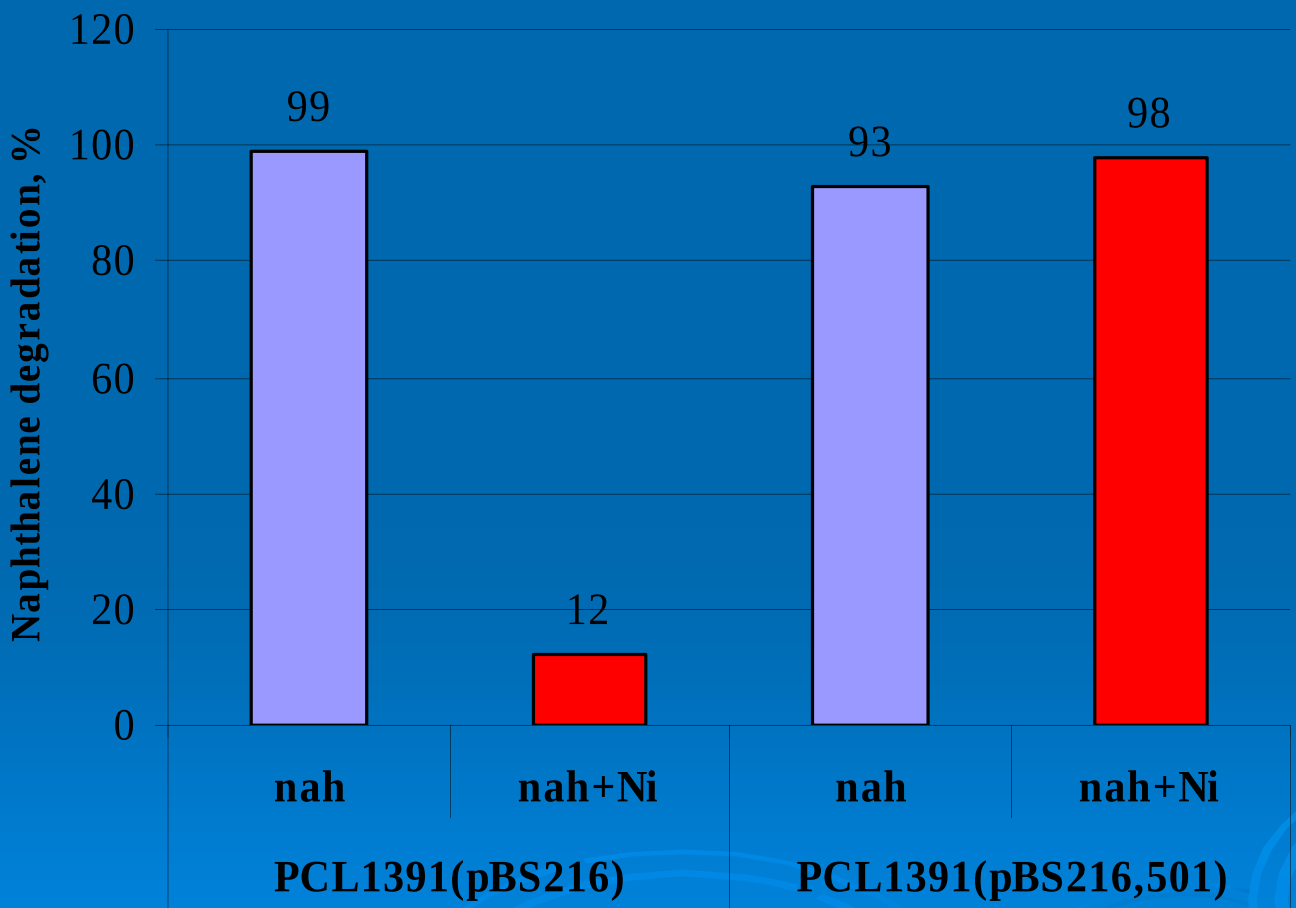
**PAH degradation**



# The construction of multifunctional PGPR *Pseudomonas*



# The naphthalene degradation by multifunctional *P. chlororaphis* strain PCL1391 (right columns) under cultivation on naphthalene and naphthalene+nickel (100 mkM)



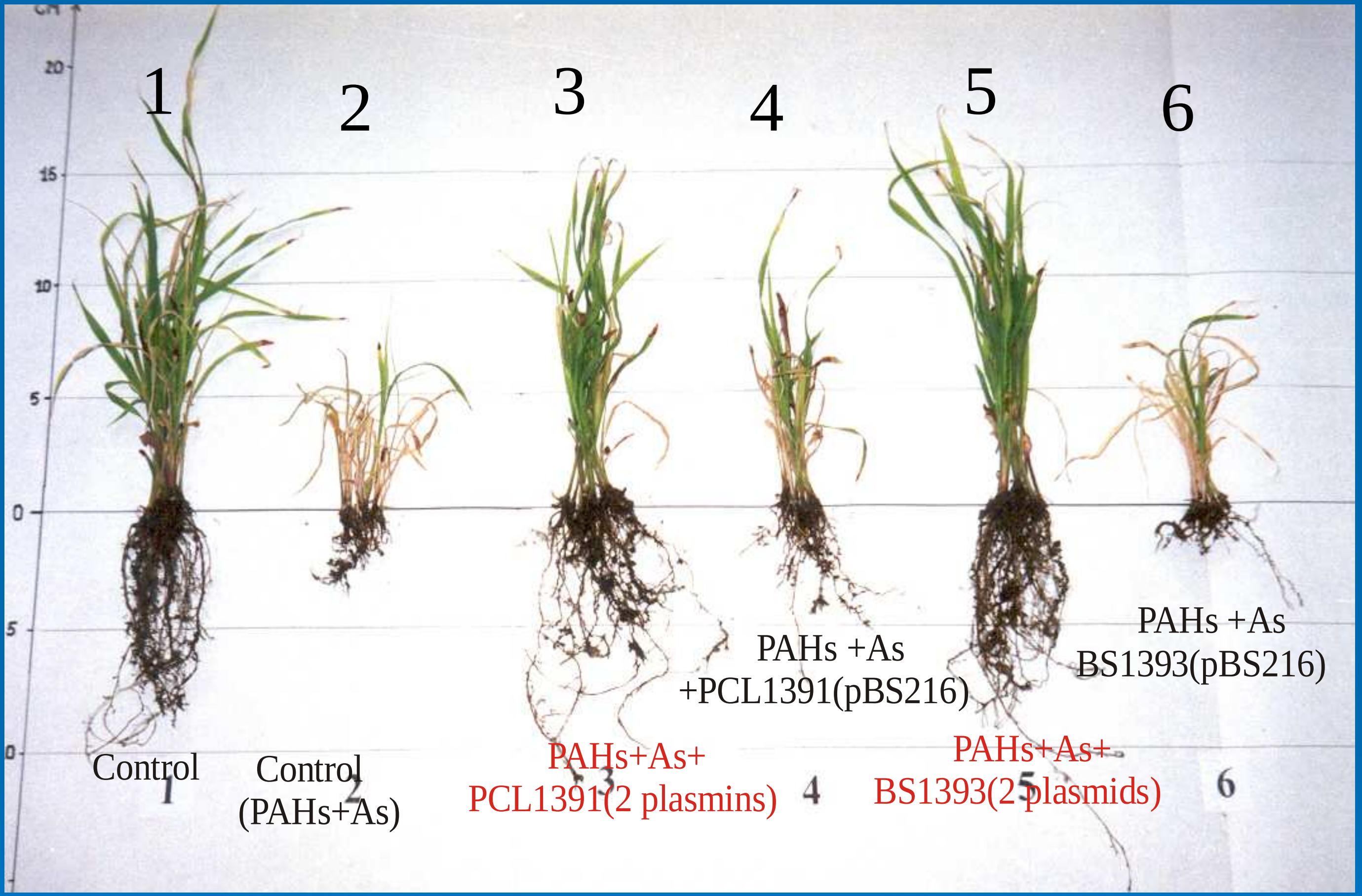
# Effect of PGPR *Pseudomonas* strains on growth of sorghum plants on soil co-contaminated with PAHs (1g/kg naphthalene+0,2g/kg phenanthrene) and nickel (400mg/kg)


**1 – no bacteria, PAHs+Ni; 2 – no bacteria, clean soil;**
**3 – PCL1391(pBS216), clean soil; 4 - PCL1391(pBS216,pBS501), clean soil;**
**5 - PCL1391(pBS216), PAHs+Ni; 6 - PCL1391(pBS216,pBS501), PAHs+Ni**

# Specific activities of the key enzymes of naphthalene degradation (cultivation on medium with naphthalene and arsenic)

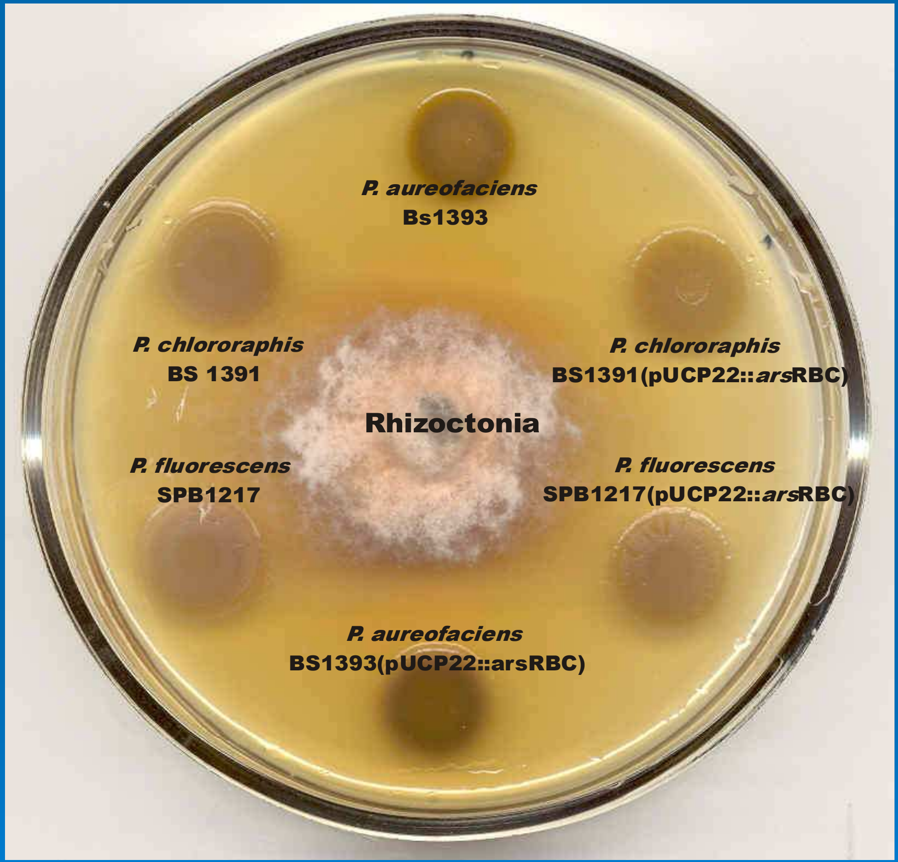
| Enzyme | OD | Specific activity, n mol/min* 1mg of protein | | | |
|---|---|---|---|---|---|
| | | BS1393 (pBS216) | PCL1391 (pBS216) | BS1393 (pBS216, arsRBC) | PCL1391 (pBS216, arsRBC) |
| NO | 0,3 | 8.5 | 12.1 | 6.2 | 11.2 |
| | 0,6 | 0 | 0 | 3.1 | 5.7 |
| SH | 0,3 | 8.5 | 4.1 | 16.4 | 15 |
| | 0,6 | 0 | 0 | 1.2 | 1,5 |
| C12O | 0,3 | 1.6 | 5.6 | 28.4 | 13.8 |
| | 0,6 | 1.1 | 3.1 | 1.15 | 3.5 |
| C23O | 0,3 | 0 | 0 | 0 | 0 |
| | 0,6 | 0 | 0 | 0 | 0 |

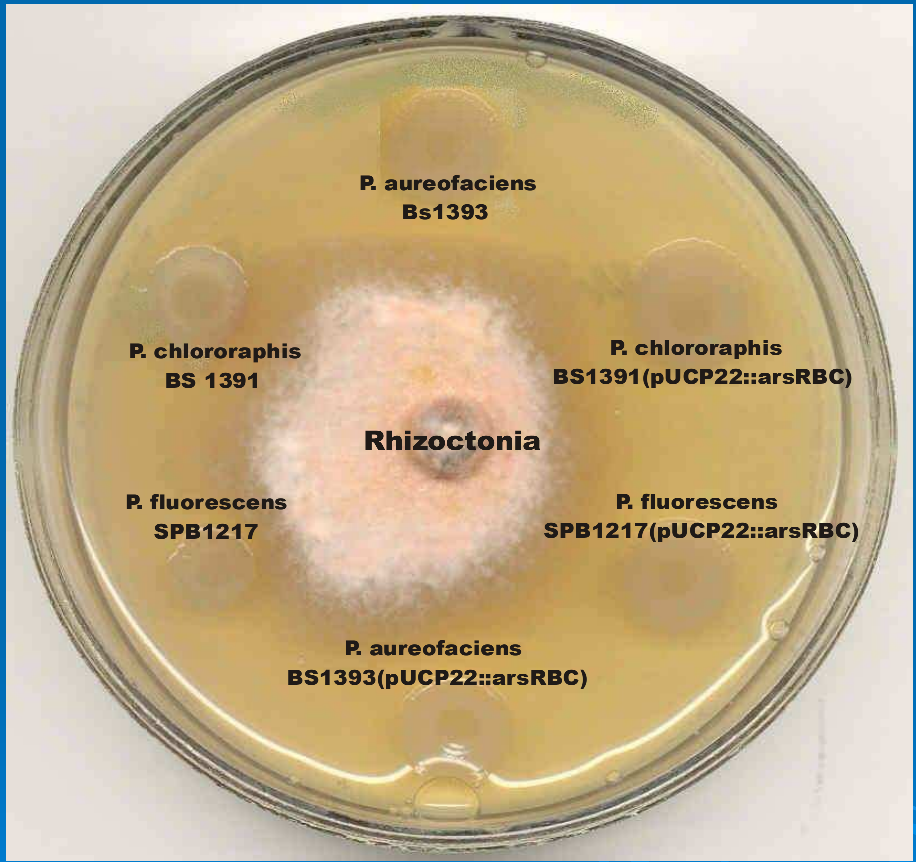
# Effect of PGPR *Pseudomonas* strains on growth of sorghum plant on soil co-contaminated with PAHs (1g/kg naphthalene+0,2g/kg phenanthrene) and arsenic (50mg/kg)




1 – no bacteria, clean soil; 2 – no bacteria, PAHs+As;
3 – PCL1391(pBS216 ,pUCP22::arsRBC), PAHs+As;
4 - PCL1391(pBS216), PAHs+As;
5 – BS1393(pBS216,pUCP22::arsRBC), PAHs+As;
6 – BS1393(pBS216), PAHs+As

# The presence of plasmids didn't affect on biocontrol properties of multifunctional strains




- Suppression *in vitro* of the phytopathogenic fungus *Rhizoctonia solani* by the various PGPR *Pseudomonas* strains
- Left- the culture medium without arsenic;
- Right – the concentration of arsenic 500 mkg/ml

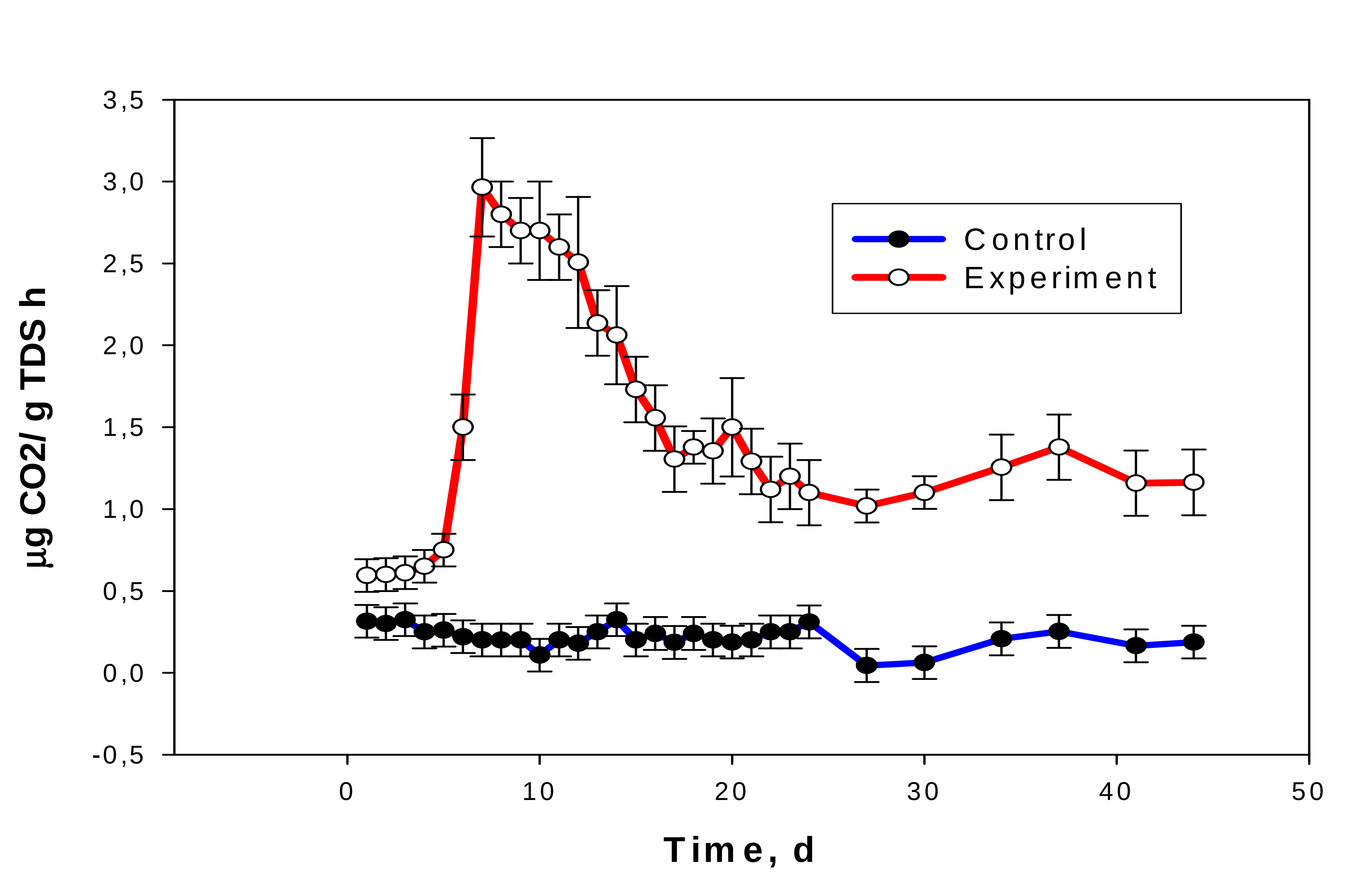
# FIELD TESTS ON OPTIMIZATION OF PHYTOREMEDIATION IN SOUTHERN RUSSIA (Abinskaya site, April 2005 – May 2007)

**Krasnodar Forestry Enterprise;**
**Institute of Biochemistry and Physiology of Microorganisms RAS, Pushchino**





- The possibility to use the stable isotope techniques to estimate the effect of crude oil pollution on soil organic matter degradation was studied.

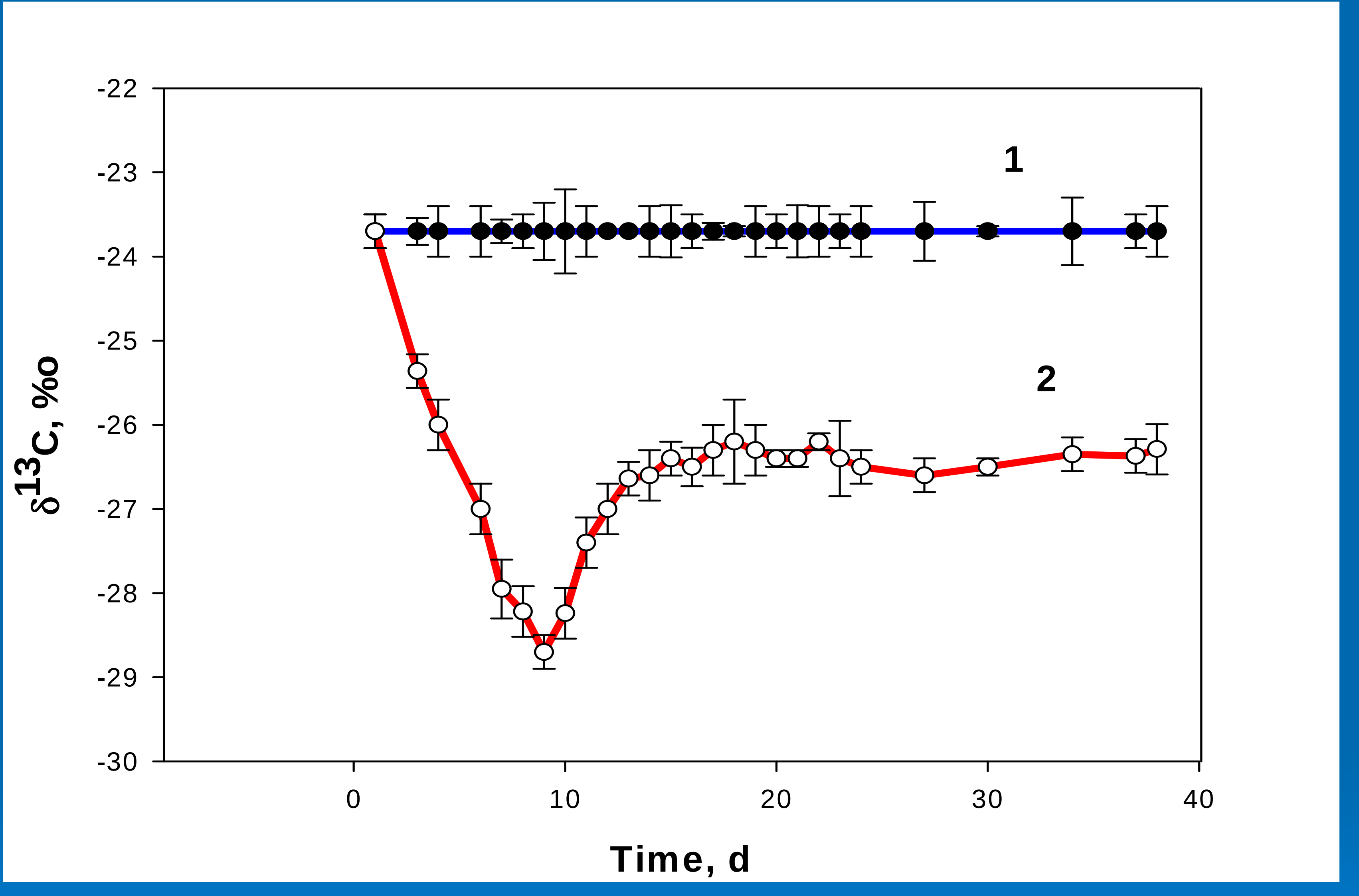



Respiration rate after crude oil addition of 3.8 % total dry soil (TDS) to an agricultural soil

The average respiration rates of $CO_2$ and total C-$CO_2$ production in control and after crude oil addition (experiment) during 47 days of exposition.

| | Rate of $CO_2$ µg C-$CO_2$ $g^{-1}$TDS $h^{-1}$ | *Total production mg C-$CO_2$ |
|---|---|---|
| **Control** | **0.228** | **25.72** |
| **Experiment** | **1.480** | **166.94** |

*Total production = Rate of $CO_2$ x Time

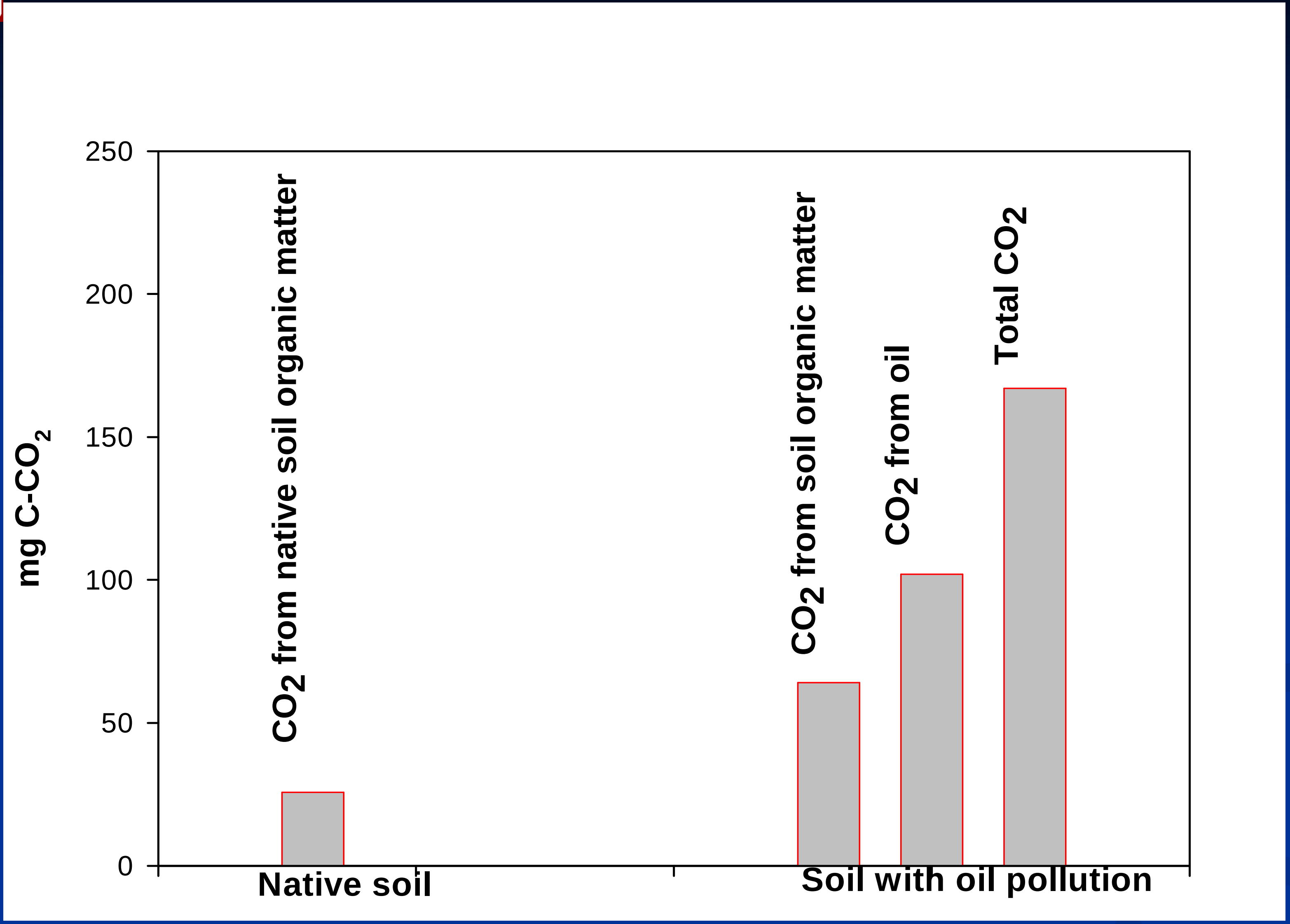



- The $\delta^{13}C$ values which relates $^{13}C/^{12}C$ isotope ratios in the metabolic $CO_2$ samples: (1) control; (2) after crude oil addition to the agricultural soil

The average carbon isotopic characteristic (**$\delta^{13}C$**) of $CO_2$ produced during soil organic matter (SOM) mineralization by soil microbiota and priming effect (PE) in control and after crude oil addition (experiment) during 47 days of exposition.

| | **$\delta^{13}C_{av}$, ‰** | ***F** % | **$^{**}[CO_2]_{SOM}$** mg C-$CO_2$ | ***** PE, %** |
|---|---|---|---|---|
| **Control** | **-23.7** | **100** | **25.72** | **0** |
| **Experiment** | **-26.59** | **38.5** | **64.30** | **150.0** |

- $\delta^{13}C_{av} = (\sum qi, \cdot \delta^{13}Ci)/\sum qi$, ‰
- $^{*}F_{SOM} = 100\ (\delta^{13}C_{exp} - \delta^{13}C_{oil})/(\delta^{13}C_{SOM} - \delta^{13}C_{oil})$
- $^{**}[CO_2]_{SOM} = [CO_2]_{total} \times F_{SOM}$
- $^{***}PE = 100 \times ([CO_2]_{SOM} - [CO_2]_{control})/[CO_2]_{control}$, %




- Quantities of microbial $CO_2$ emission from arable soil with and without the crude oil pollution determined by isotopic ratio mass spectrometry (IRMS)

- The total $CO_2$ emission from oil-containing soil samples to atmosphere during 47 days of observation was 6.8-fold more then from native soil.
- At the same time, the amount of metabolic $CO_2$ produced due to soil organic matter mineralization was about 38 % of the total $CO_2$ flow and that due to utilization of oil hydrocarbons reached 62 %.

# CONCLUSIONS

- **The introduction of both degradative plasmids and plasmids of resistance to toxic compounds into PGPR *Pseudomonas* conferred them the ability to degrade PAH in the presence of these compounds.**

- **The model experiments on the soils co-contaminated by PAH mixture (naphthalene - 1 g/kg of soil and phenanthrene - 0.2 g/kg), nickel (400 mg/kg) and arsenic (50 mg/kg) have demonstrated that some plasmid bearing PGPR strains protected plants both from the soil-borne plant pathogens and from the toxic compounds very efficiently.**

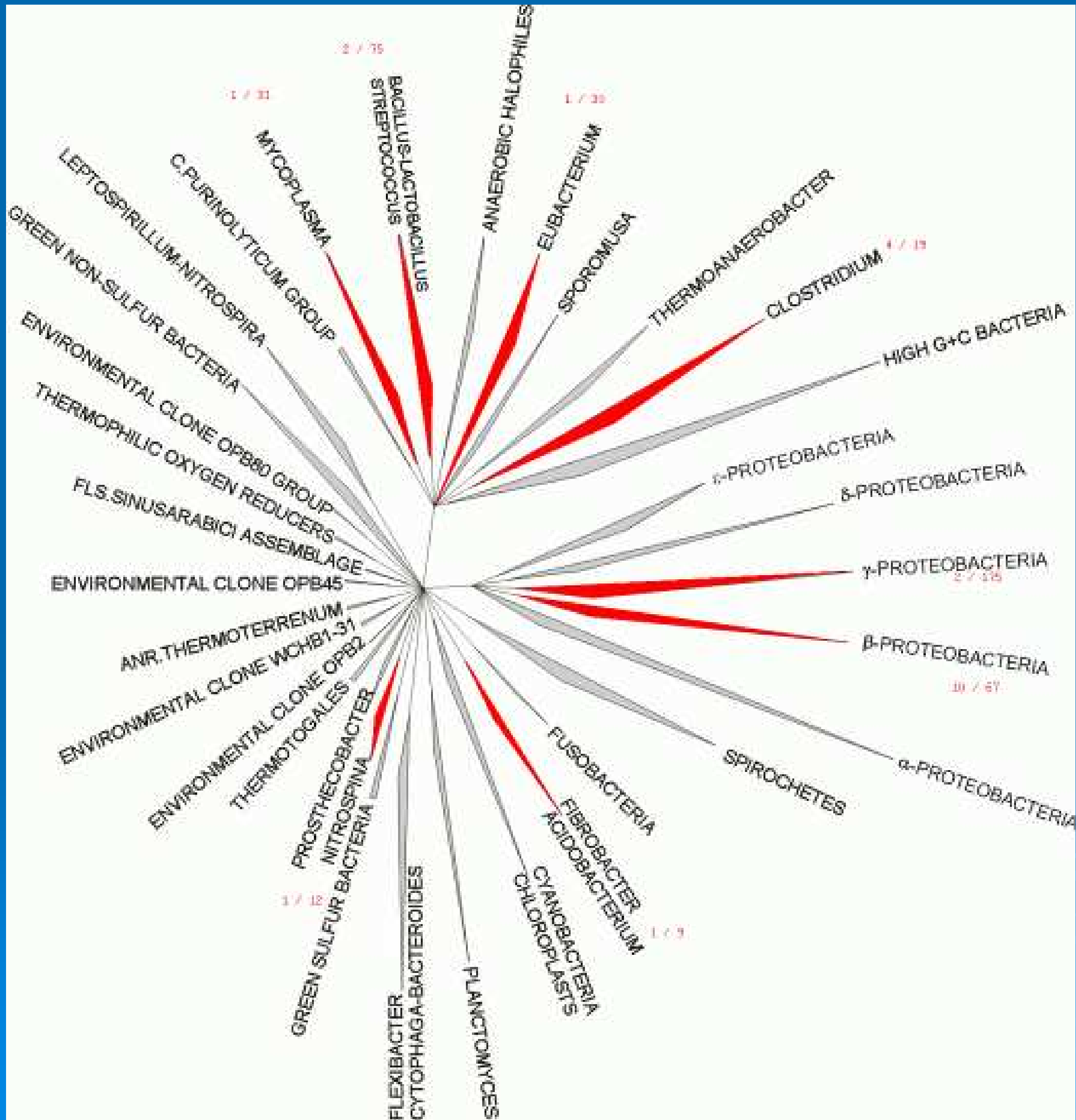
- **Combination of degradative potential of plant growth-promoting rhizobacteria with plants for phytoremediation demonstrated the effectiveness of this approach.**

- **The multifold increase of production of carbon dioxide, which is released to atmosphere is a result of microbial processes in oil-polluted soil.**

② ACKNOWLEDGEMENTS:

- **The work is supported by**
- **1) CRDF grant # RUB2-010001-PU-05;**
- **2) Ministry of Education and Science of Russian Federation grant #RNP 2.1.1.9321;**
- **3) Program #16 of Basic Research of the Presidium of the Russian Academy of Sciences;**
- **4) Goal-Oriented Program of the Presidium of the Russian Academy of Sciences "Support of Innovations".**

# Diversity of Microorganisms from Oil Slimes



# Diversity of Microorganisms from Oil Slimes

THERMUS THERMOPHILUS_SUBGROUP
THERMUS THERMOPHILUS_SUBGROUP
THERMUS THERMOPHILUS_SUBGROUP
AZOSPIRILLUM
LIPOFERUM_SUBGROUP
METHYLOSINUS
TRICHOSPORIUM_SUBGROUP
RHODOFERAX
FERMENTANS_SUBGROUP
RHODOFERAX
FERMENTANS_SUBGROUP
ACIDOVORAX AVENAE_SUBGROUP
ACIDOVORAX AVENAE_SUBGROUP
AQUASPIRILLUM
PSYCHROPHILUM_SUBGROUP
BURKHOLDERIA GLATHEI_SUBGROUP
BACILLUS
STEAROTHERMOPHILUS_SUBGROUP
LEPTOSPIRILLUM
FERROOXIDANS_SUBGROUP
METHYLOSINUS
TRICHOSPORIUM_SUBGROUP
METHYLOBACTERIUM
ORGANOPHILUM_SUBGROUP
IODOBACTER FLUVIATILE
NEISSERIA
GONORRHOEAE_SUBGROUP
NEISSERIA
GONORRHOEAE_SUBGROUP
NEISSERIA
GONORRHOEAE_SUBGROUP

NEISSERIA
GONORRHOEAE_SUBGROUP
NITROSOMONAS
EUROPAEA_SUBGROUP
RHODOFERAX
FERMENTANS_SUBGROUP
CLOSTRIDIUM
THERMOBUTYRICUM_SUBGROUP
CHROMOBACTERIUM
VIOLACEUM_SUBGROUP
NEISSERIA
GONORRHOEAE_SUBGROUP
NEISSERIA
GONORRHOEAE_SUBGROUP
NEISSERIA
GONORRHOEAE_SUBGROUP
NEISSERIA
GONORRHOEAE_SUBGROUP
VARIOVORAX PARADOXUS_SUBGROUP
BURKHOLDERIA GLATHEI_SUBGROUP
DEHALOCOCCOIDES
ETHENOGENES_SUBGROUP
CLOSTRIDIUM BOTULINUM_SUBGROUP
CLOSTRIDIUM BOTULINUM_SUBGROUP
CLOSTRIDIUM
COCHLEARIUM_SUBGROUP
CLOSTRIDIUM
COCHLEARIUM_SUBGROUP
CLOSTRIDIUM
AURANTIBUTYRICUM_SUBGROUP
HYDROGENOPHILUS
THERMOLUTEOLUS_SUBGROUP

COMAMONAS TERRIGENA_SUBGROUP
RHODOFERAX
FERMENTANS_SUBGROUP
BURKHOLDERIA GLATHEI_SUBGROUP
ACHROMATIUM_ASSEMBLAGE
ACHROMATIUM_ASSEMBLAGE
GEOBACTER
METALLIREDUCENS_SUBGROUP
CLOSTRIDIUM
AURANTIBUTYRICUM_SUBGROUP
CLOSTRIDIUM
AURANTIBUTYRICUM_SUBGROUP
MESORHIZOBIUM LOTI_SUBGROUP
NITROSOSPIRA
MULTIFORMIS_SUBGROUP
NITROSOSPIRA
MULTIFORMIS_SUBGROUP
HYDROGENOPHILUS
THERMOLUTEOLUS_SUBGROUP
COMAMONAS TERRIGENA_SUBGROUP
COMAMONAS TERRIGENA_SUBGROUP
ACIDOVORAX AVENAE_SUBGROUP
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ACIDOVORAX AVENAE_SUBGROUP
BURKHOLDERIA GLATHEI_SUBGROUP
ACHROMATIUM_ASSEMBLAGE
CLOSTRIDIUM
ALGIDICARNIS_SUBGROUP
BURKHOLDERIA CEPACIA_SUBGROUP

# Lysis of cianobacteria by ultramicrobacteria